Antimicrobial Peptide Exposure and Reduced Susceptibility to Daptomycin: Insights Into a Complex Genetic Puzzle

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(See the major article by Mishra et al, on pages 1160–7.)

Adequate antibiotic treatment of bacterial infections is one of the most important problems of our time, not only because of the incessant development of antibiotic resistance, but also because of diminished development of novel antibacterial products. Attention has been focused on this problem by the recent United Nations World Health Day in April 2011, which highlighted antibiotic resistance and issued calls for urgent action by expert scientific panels tasked to establish priorities and solutions [1]. Among the proposals is a call for enhanced understanding of resistance, including mechanisms and means of predicting its emergence. Obtaining this knowledge will clearly affect the prudent use of antibiotics and drive novel antibiotic discovery and development strategies.

Cationicantimicrobialpeptides(CAMPs) are compounds of great pharmacologic and biologic interest, especially because they have nonspecific and multiple modes of action, which are believed to thwart or forestall the development of resistance. CAMPs are widely distributed in nature and constitute key effectors in innate immune responses to infection in organisms ranging from mammals to plants. CAMPs share cationic and amphipathic properties but vary in sequence, secondary structures, and size. Their antimicrobial activity is initiated through a nonspecific electrostatic interaction with the anionic heads of membrane phospholipids, leading to membrane depolarization or pore formation. Increasing evidence indicates that some CAMPs are probably internalized, leading to interaction with intracellular targets, suggesting that membrane damage alone might not be the principal antimicrobial mechanism of CAMPs [2–4].

Although CAMPs are considered promising candidate templates for development of novel antimicrobials, it has recently been shown that bacteria are capable of adapting and resisting CAMPs, perhaps because of co-evolution within their host. These resistance mechanisms include production of peptidases and proteases that degrade antimicrobial peptides, production of compounds that inhibit the action of CAMPs, and reduction of net anionic charge of the bacterial cell envelope [5].

Daptomycin, a calcium-dependent antimicrobial lipopeptide, is used to treat certain skin infections resulting from various gram-positive organisms and especially bloodstream infections due to *Staphylococcus aureus*. In some respects, daptomycin resembles CAMPs because of its peptide content, charge, and mode of action targeting membrane function. *S. aureus* strains displaying reduced susceptibility to daptomycin have been observed both in vivo and in vitro [6–8]. Of interest, cross-resistance between daptomycin and other CAMPs that target the bacterial cell membrane has also been reported. These studies suggest that exposure to daptomycin could confer reduced susceptibility to endovascular host defense antimicrobial peptides, notably thrombin-induced platelet microbicidal proteins (tPMPs) and human neutrophil peptide-1 (hNP-1) [9]. A natural extension of these findings concerning cross-resistance evolution is to consider the consequences of the reciprocal order of exposure.

In this issue of the *Journal*, Mishra and coworkers address this important aspect by asking whether there is a potential priming role of preexposure to endovascular host cationic peptides in the development of early stages of bacterial resistance to daptomycin. Their study relied on a carefully selected set of 47 independent methicillin-resistant *S. aureus* (MRSA) strains collected from

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bacteremic episodes, excluding isolates obtained from patients with clinical evidence of endocarditis. Of importance, the archived strain set chosen for the study predated the commercial introduction of daptomycin in 2003, and all strains were, therefore, deemed "daptomycin naive." Since exposure to vancomycin can evoke measurable changes in daptomycin susceptibility, the authors were also careful to test the MRSA strain set for vancomycin susceptibility. All strains were vancomycin susceptible.

As a first step, in vitro assays established that bacterial survival following exposure to tPMP and hNP-1 was strongly correlated. In other words, an increased survival after exposure to one peptide was statistically linked to increased survival after exposure to the other peptide in this MRSA strain cohort. Given this correlation, they next asked whether strains showing increased survival to host cationic peptides concomitantly displayed altered daptomycin-susceptibility profiles. A positive correlation would provide evidence that prior exposure to these peptides could conceivably drive selection of daptomycin-resistant strains. Indeed, the authors found that higher daptomycin MICs tracked with increased resistance to killing by one host defense peptide, tPMP, suggesting that exposure to CAMPs could drive selection of strains with reduced susceptibility to daptomycin. These findings are in accordance with previous observations that genetic changes responsible for reduced susceptibility to daptomycin are also implicated in resistance to CAMPs. These changes involve reported alterations in membrane fluidity, thickened cell-wall, and increased positive cell-wall charge [9–11].

A significant point to emerge from the present study involved data exposing the possibility that *S. aureus* encounters with an endovascular platelet-derived CAMP could conceivably prime altered susceptibility to daptomycin and evoke a phenomenon similar to the so-called MIC creep described for vancomycin. MIC creep is an important issue to pursue in detail, especially with the documented tendency of vancomycin MIC creep to correlate with suboptimal treatment outcomes [12]. Whether this phenomenon occurs with daptomycin will be an important issue to address in future studies. An additional point raised in the study by Mishra et al was the hypothesis that the ability of *S. aureus* to survive within the bloodstream might depend, in part, on the acquisition of resistance to tPMP killing.

S. aureus traveling in the bloodstream will encounter a range of host defense mechanisms, and it is reasonable that these conditions represent a robust environment driving genetic selection. Indeed, one of the hallmarks of *S. aureus* is its impressive arsenal of defense mechanisms that allow it to deter the complement, opsonization, reactive oxygen species, and iron limitation [13–15]. Even selective pressure by host lysozyme has driven the establishment of acquired resistance to this enzyme in all tested pathologic strains of staphylococci [16, 17].

Studies such as this one underscore the need to understand how bacteria acquire reduced susceptibility to CAMPs, compared with how reduced susceptibility to daptomycin occurs. The genetic changes driving resistance to daptomycin are thought to result in altered physiochemical properties of the cell membrane fluidity and surface electrostatic charge. By enhancing net surface positive charge through several mechanisms, notably via reactions catalyzed by MprF and DltA, cationic molecules would be repulsed and would be unable to access the bacterial membrane efficiently. Alterations in MprF, a bifunctional synthase/translocase responsible for lysinylation of phosphatidylglycerol, results in a gain of positively charged phospholipid and, thus, enhanced positive surface membrane charge, whereas DltA, an enzyme that adds alanine to polyanionic teichoic acid chains, reduces net negative surface charge. Altered resistance to cationic antimicrobial peptides also appears to be mediated, in part, by MprF and DltABCD-mediated surface modifications [18]. Reports to date would, therefore, suggest that there is reason to expect some mechanistic overlap that would explain the phenomenon of cross-resistance in molecular detail. A key to understanding this linkage is the growing body of evidence linking mutation of the histidine kinase 2-component sensor system GraRS (also called aps) to changes in susceptibility to CAMPs, daptomycin, and glycopeptides.

A search for genes whose overexpression affected glycopeptide resistance in *S. aureus* first suggested a role for *graRS* [19]. Subsequent study indicated that point mutations within the gene encoding GraRS could dramatically affect conversion of heterogeneous vancomycin-intermediate *S. aureus* to intermediate vancomycin resistance [20, 21], and phenotypic analysis suggested that mutation in graR could also affect daptomycin susceptibility [20]. Concurrent studies designed to uncover mechanisms governing susceptibility to various antimicrobial peptides in both *Staphylococcus epidermidis* and *S. aureus* showed an important role of GraRS [16, 22–25]. Extensive gene expression analysis has demonstrated that GraRS controls a large regulon in *S. aureus,* but that, most notably, it modulates the expression of *mprF* and the *dltABCD* operon involved in the controlling bacterial surface charge [16, 24, 25–27]. Of interest, only certain CAMPs can activate the GraRS system, which in turn increases resistance to these specific CAMPs [23, 28]. However, resistance to other CAMPs is observed even in the presence of non-inducible GraRS CAMP molecules, suggesting that there are both GraRS-dependent and -independent CAMP resistant pathways [26].

Although the results presented by Mishra et al in the present issue raise some tantalizing possibilities, it is important to consider the findings as preliminary and, as the authors point out, subject to a number of caveats. For instance, the MRSA cohort could

comprise fortuitously strains harboring reduced susceptibility to certain CAMPs, which may not be a feature found in other independent cohorts. Second, the conditions chosen for in vitro study of CAMP susceptibility used a limited range of peptide concentrations and were performed in the absence of serum. Third, a limited set of CAMPs was tested (2), and it is certainly possible that in the context of infection, bacteria are exposed to a broad range of CAMPs issued from various host-cell responses.

It is curious that enhanced resistance to killing by only 1 of the 2 CAMPS studied (ie, platelet-derived tPMP) correlated with reduced daptomycin susceptibility, whereas the neutrophil defensin hNP-1 was not correlated. Previous work had shown that the mechanisms of action of tPMP and hNP-1 were different [29], and, thus, one plausible explanation could be simply that platelet-derived tPMP was a more robust stimulus driving bacterial sensory systems. It is intriguing that, in support of this possibility, recent work now shows that hNP-1 exposure in vitro does not induce *mprF* or *dltA,* as judged by findings of quantitative reverse-transcription polymerase chain reaction assay [28].

Additional observations revealed in the study by Mishra et al warrant mention. The authors noted no significant correlation with *agr* clonotype but, nevertheless, noted a strong association between certain *agr* types and susceptibility to killing by host defense peptides. Furthermore, despite demonstrating that all tested strains were susceptible to vancomycin, a large proportion of the cohort was isolated from patients who had received intravenous vancomycin ≤30 days before the documented bloodstream infection. This circumstance raises the obvious question whether strains could have acquired heritable genetic traits following vancomycin exposure that did not overtly affect measurable vancomycin MIC but could nonetheless affect reduced susceptibility to daptomycin.

It is clear that future studies will involve determining precisely how exposure to various host defense peptides imparts a selective advantage to *S. aureus* to promote infection and disease. In addition, it will be imperative to determine whether and to what extent altered resistance to host defense peptides alters the efficacy of daptomycin. Of importance, the mounting evidence of antimicrobial resistance mechanisms shared among CAMPs, daptomycin, and glycopeptides emphasizes the need to pursue detailed knowledge of these mechanisms, because studies are convincingly showing that we face far more complex drug-resistance patterns than previously imagined.

Notes

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